



Analgesic activity of new complex compounds SnCl_4 with salicyloylhydrazones of benzaldehyde and brombenzaldehyde

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The physiological role of pain helps to avoid or limit the tissue damage and therefore it provides the vital protective function. It has been previously studied, that complex compounds SnCl_4 with salicyloylhydrazones of benzaldehyde and brombenzaldehyde as well as their components represent the practical interest by the manifestation of anti-inflammatory and antidepressive action. In this case the analgesic properties of the complexes themselves, as well as compounds belonging to their structure were of interest of our study. The experiments were performed on white mice. Several chemical (allyl isothiocyanate or AITC, capsaicin, formalin) and thermal effects have been chosen in this investigation for studying the analgesic activity and establishing the possible mechanisms of antinociceptive effect. The tested compounds showed a mixed type of receptor interaction using models with different types of stimulation, which allows to make conclusions about their perspective as highly effective analgesics with a complex mechanism of action.

INTRODUCTION

Many acute and chronic diseases, medical manipulations and injuries are closely related to pain. The pain is one of the most important and main functions of the body that mobilises various functional systems to protect it from the influence of the damaging factors. The physiological role of pain helps to avoid or limit the tissue damage and therefore it provides the vital protective function (1). The chemical and physical impacts occurring outside of the nervous system cause the irritation of the pain receptors – nociceptors (free nerve endings). Nociceptors conduct electrical impulses using the physiological healthy nervous system by stimulating the activity of the cerebral cortex thereby causing the feelings of pain (2). Pain is not limited by organic or functional disorders in the site of its localization, but also negatively affects the general and emotional health. Pain that continues for a long time leads to many organic and mental disorders. It is known that chronic pain syndrome and depressive disorders have common neurochemical origin. Data showed that antidepressants that were prescribed to patients without depressive disorders also led to the pain reducing (3,4).

In this case the studied complex compounds SnCl_4 with salicyloylhydrazones of benzaldehyde and brombenzaldehyde as well as their components represent the practical interest by the manifestation of different pharmacological action (anti-inflammatory (5) and antidepressive (6)), therefore their usage in the complex therapy against

various diseases and finding the methods of their inoculation have a potential interest in further treatment. Fact that salicylic acid that relate to salicylates had the anti-inflammatory properties. Its mechanism of action is ability to inhibit the prostaglandins synthesis and specifically inhibition of cyclooxygenases (COXs) (7). It is known that salicylic acid has the most pronounced anti-inflammatory effect at concentrations from 0.5% to 5% (8,9). Also, previous studies of our colleagues were focused on some pharmacological properties of hydrazones (the organic compounds, $\text{R}_1\text{R}_2\text{C} = \text{NNH}_2$ (10) – their structural formula), and it was proved that hydrazones had shown anti-inflammatory, analgesic, antispasmodic and other activities (11). Hydrazone active sites (Carbon and Nitrogen) are responsible for their physical and chemical properties and they are used for synthesizing new organic compounds through their reactivity. In this study both for acute and inflammatory pain therapy the subcutaneous injection of tested compounds has been used. This injective method helped, on the one hand, to deliver the medical compound to the medication site and, on the other hand, to avoid the higher concentration of the medical compound in the systemic circulation.

In this context, the analgesic properties of the complexes themselves, as well as compounds belonging to their structure were of interest of our study.

Several chemical (allyl isothiocyanate or AITC, capsaicin, formalin) and thermal effects have been chosen in this investigation for studying the analgesic activity and establishing the possible mechanisms of antinociceptive effect.

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MATERIALS AND METHODS

The experiments were performed on white mice with weight around 18-20 g, animals were obtained from the vivarium of Odessa National Medical University. During the experiment all animals were kept in vivarium conditions on the standard diet with free access to water and nutrition.

All studies conformed to the rules of the “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (12) (Strasbourg, 1986) and the principles of the Ukrainian National Congress on Bioethics (13).

Mice were divided into 3 groups (for each test) and 11 subgroups (for each test-compounds and controls) 5 animals in each subgroup. Anaesthesine ointment 2% was used as reference medicine. Complex compounds I and II, benzaldehyde, 4-brombenzaldehyde, salicyloylhydrazones of benzaldehyde (SHB) and 4-brombenzaldehyde (SHBrB), salicylic acid (SA), and mixtures of benzaldehyde with SA, 4-brombenzaldehyde with SA, and anaesthesin were applied on skin using polyoxyethylene based ointment with benzocainum 2% 15 min prior the experiment. The basis contents of polyethylene glycol-1500: polyoxyethylene-400: 1,2-propylene glycol in the ratio of 4:2:3 respectively. The control group of animals hasn't been treated.

To simulate nociceptive reaction 20 µl of 0.5% solution of AITC in 1,2-propylene glycol were subplatory injected into the hind limb. The pain response, especially the duration of licking of the damaged paw, was registered during 10 min after phlogogen inoculation. Capsaicin (alkaloid that contained in a group of plants of Capsicum genus) is A-type potassium channel blocker and antagonist of vanilloid receptors (TRPV-1). The pain response was induced by injection of 6 µl of capsaicin solution (6 µg in 20 µl of 1,2-propylene glycol) into the back surface of the mouse foot and the time of licking of the damaged paw was recorded during 5 min after the beginning of the experiment.

Advantage of the formalin test is the ability to evaluate two types of pain for a long period of time (2 stages) (14, 15). It is well known that I stage (the first 5 min after phlogogen inoculation) is the stage of acute pain and it is related to direct activation of thin unmyelinated C-fibers, most of them transfer impulse from the pain receptors. In contrast, II stage characterizes pain that is induced by inflammatory factors in the peripheric tissues, and appears after 10-15 min after the beginning of experiment and ends after 50-55 min. The oedema was induced by subplatory injection of 20 µl 2% solution in water of formalin into the animal hind limb. Animals were placed into the single cage and they were looked for an hour, recording time, during that mouse was licking its limb (16).

Also, hot plate test was used for studying of analgesic activity of tested compounds using the model of physical influence. Animal was placed on the special surface, heated up to 55 °C, and the latent time was recorded during that the pain syndrome hasn't appear. The pain was expressed in licking and pulling paws off the hot surface (17). Ointment 2% with appropriate compounds was applied on the animal limbs and the longer the mouse was on the hot surface without showing the painful effect, the more pronounced analgesic effect was in the tested compound.

Obtained data were statistically processed using Microsoft Excel tools, calculation the average value for each subgroup as well as standard error for the average. Analgesic activity was represented as the average time of licking for the chemical pain influence model. All data were calculated and represented as a percentage from the control.

RESULTS AND DISCUSSION

Models of chemical and thermal pain stimulation are distinguished by a system of nociception, transferring of the nervous impulse as well as its sensation (18, 19). The first step of our study was the investigation of nociceptive action on the chemical stimulation models. One of such stimuli is AITC (mustard oil), the selective antagonist of TRPA-1. During the TRPA-1 inhibition the Ca-mediated response is eliminated in the sensory neurons of the posterior roots and the trigeminal nerve, causing the development of acute and inflammatory pain (20, 21). Next stimulus was capsaicin that was used for analgesic effect study. Capsaicin (alkaloid that are contained in a group of plants of Capsicum genus) is A-type potassium channel blocker and antagonist of vanilloid receptors (TRPV-1). According to the obtained data (Tab. 1), tested compounds showed different analgesic activity using model of the pain response which suggest the possible mechanism of their analgesic action.

Table 1 Analgesic effect of tested compounds as a percentage from the control

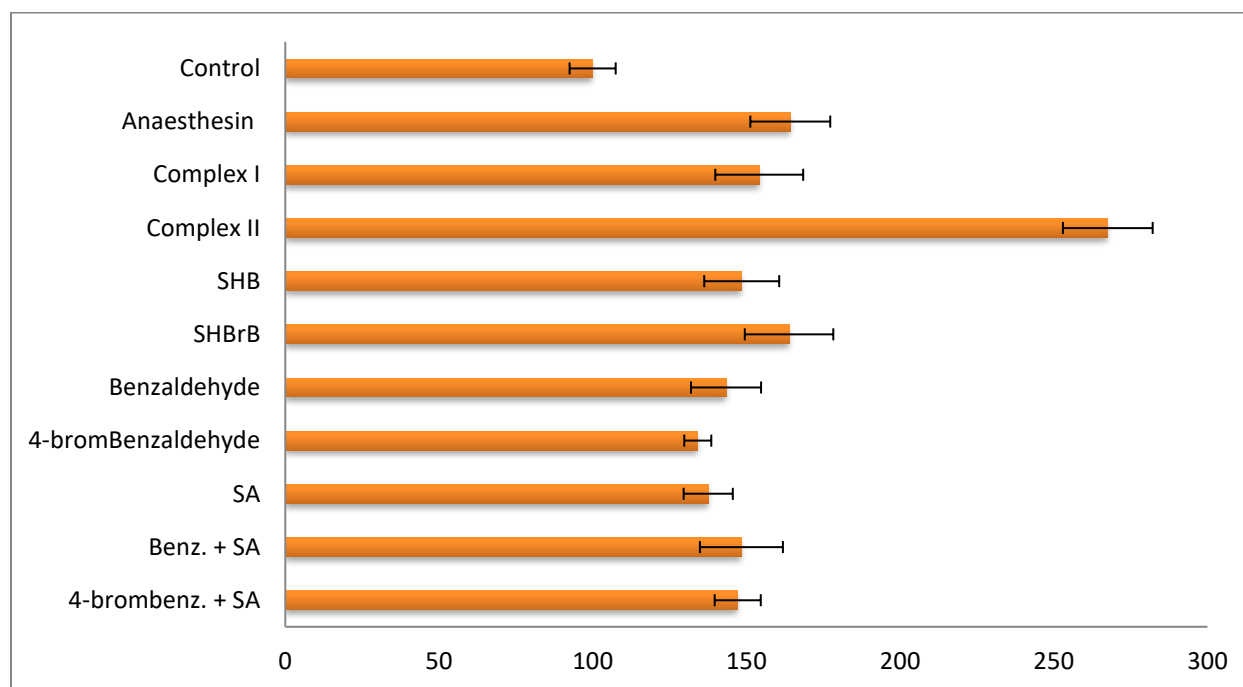
	AITC	Capsaicin
Control	100 ± 2.0	100 ± 8.0
Anaesthesin	40.9 ± 4.9	24.0 ± 1.6
Complex I	18.7 ± 5.3	10.5 ± 1.2
Complex II	44.1 ± 10.7	29.5 ± 4.4
SHB	57.3 ± 9.5	9.2 ± 0.5
SHBrB	16.6 ± 0.2	44.4 ± 3.0
Benzaldehyde	108.3 ± 3.8	34.2 ± 1.6
4-brombenzaldehyde	38.6 ± 14.5	44.1 ± 0.2
SA	32.8 ± 1.3	25.6 ± 4.9
Benz. + SA	115.1 ± 13.3	25.9 ± 4.5
4-brombenz. +SA	22.5 ± 4.4	77.0 ± 4.5

It should be noted that the least pronounced specific painful reaction in mice has been observed after applying of the salicyloylhydrazones of benzaldehyde using capsaicin inflammation model. The analgesic activity of benzaldehyde, salicylic acid as well as their mixture showed that they play a valuable role in the anesthetizing effect of the complex compound I, acting on TRPV1 receptors. The analgesic activity of benzaldehyde, salicylic acid was realized by increasing of latent time of the pain response developing and decreasing the duration of the paw licking by 70% comparing to control in average. Interestingly that analgesic effect of benzaldehyde as well as benzaldehyde and SA mixture hasn't been observed using a model of pain stimulation induced by AITC. It might indicate that tested compounds in this combination has not impact on TRPA1 receptors. It is obvious that complex compound I showed its analgesic effect using test model with AITC due to the presence of the residue of salicylic acid molecule in the structure.

Salicyloylhydrazone of 4-brombenzaldehyde, 4-brombenzaldehyde as well as 4-brombenzaldehyde and SA mixture showed analgesic effect both on inflammatory models, but level of their activity was different comparing to the reference medicine anaesthesin. Thus, during the inflammation caused by capsaicin salicyloylhydrazone of 4-brombenzaldehyde, 4-brombenzaldehyde as well as 4-brombenzaldehyde and SA mixture had worse analgesic effect comparing to anaesthesin and it was 44.4% and 25.6% prospectively. Obtained data as a result of the test on the model of inflammation caused by AITC showed a pronounced analgesic effect of the mixture of 4-brombenzaldehyde and SA, representing 22.5%. The most pronounced anaesthetic effect was observed in the salicyloylhydrazone of 4-brombenzaldehyde as a response to AITC inoculation (decreasing

Table 2 Analgesic effect of tested compounds as a percentage from the control

	Formalin	
	1 stage	2 stage
Control	100 ± 7.9	100 ± 6.3
Anaesthesin	55.1 ± 4.1	60.9 ± 7.1
Complex I	51.0 ± 5.4	22.1 ± 0.1
Complex II	83.5 ± 4.6	28.4 ± 10.1
SHB	94.9 ± 7.3	0.0
SHBrB	88.2 ± 7.9	30.4 ± 6.2
Benzaldehyde	121.2 ± 4.6	4.8 ± 1.6
4-bromBenzaldehyde	113.8 ± 8.0	23.8 ± 15.0
SA	90.2 ± 15.0	12.6 ± 3.8
Benz. + SA	122.0 ± 15.8	16.9 ± 11.2
4-brombenz. + SA	98.9 ± 8.0	12.0 ± 5.7

**Graph 1**

Analgesic effect of tested compounds as a percentage from the control HP-test

number of licking of the damaged limb by 24% comparing to the reference medicine). Analgesic activity of the complex compound II was equal to the compared medicine – anaesthesin. The high analgesic activity of the compounds in two tests suggests the activation of several types of receptors, in particular TRPV1 and TRPA1.

It is known that formalin test allows to differentiate different mechanisms of analgesic activity of medical compounds and it was proved that in the acute stage the compounds haven't shown the activity with a preferential mechanism of action was the impact on the COX-2 synthesis with subsequent suppression of prostaglandin production, thereby reducing the inflammation (22).

Earlier, on a model of carrageen an inflammation, it was proved that complex compounds I and II, as well as benzaldehyde and 4-bromobenzaldehyde that belong to those complexes, have an anti-inflammatory effect, confirmed by the data obtained as a result of the formalin test, specifically the pain reduction in the second (inflammatory) stage of the experiment (23).

Obtained results from the first stage of the formalin inflammation (Tab. 2.) indicated that all tested compounds had almost no analgesic effect in this stage, which was probably due to the absence of the effect of the studied compounds on the peripheral nociceptors.

SHB 2% ointment had the most pronounced analgesic effect in the first stage of formalin inflammation – the animals completely ignored the inflamed limb, while after applying 2% benzaldehyde ointment, the number of licks was 4.8% comparing with the control group. The obtained data suggested that the analgesic effect of these compounds in this case was caused by their inhibitory effect on the activity of cyclooxygenase.

The second stage of this study was the investigation of nociceptive action on a model of physical stimulation, for which "hot plate" test (HP-test) has been chosen. HP-test allows to evaluate the possible mechanisms of action of the studied medical substances and their impact on TRPV1 receptors (24). According to the obtained data, in the HP-test all compounds successfully reduced the level of pain sensitivity (Graph. 1). After the inoculation of all tested compounds, a significant increase

in the latent time of the animal's response to superficial and acute pain was observed on average by 34%-64%.

The most pronounced level of the painful activity inhibition was shown by complex compound II, which probably might indicate the involvement of TRPV1 receptors.

CONCLUSION

All compounds that were used in the experiment showed analgesic activity. Salicyloylhydrazones both of benzaldehyde and 4-brombenzaldehyde have a more pronounced analgesic effect than benzaldehyde/ 4-brombenzaldehyde mixtures with SA and these substances separately. The tested compounds showed a mixed type of receptor interaction using models with different types of stimulation, which allows to make conclusions about their perspective as highly effective analgesics with a complex mechanism of action.

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Author's Participation

Elena Prokopchuk (E.P.), Alexandra Aleksandrova (A.A.) and Iryna Kravchenko (I.K.) conceived and planned the experiments. E.P. and A.A. carried out the experiments. E.P., A.A. and I.K. contributed to the interpretation of the results. E.P. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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